

ORIGINAL RESEARCH ARTICLE

Regulatory role of lncRNA MALAT1 and miR-150-3p interaction in breast cancer progression and therapeutic implications

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Abstract

Long non-coding RNAs (lncRNAs) play crucial roles in cancer progression and metastasis. However, the precise regulatory mechanisms of MALAT1, an lncRNA, in breast cancer remain elusive. Consequently, this study explored the specific role of MALAT1 as a tumor promoter in breast cancer. Initially, MALAT1-small interfering RNA (siRNA) transfection resulted in the inhibition of breast cancer cell migration, colony formation, and invasion. Real-time polymerase chain reaction analysis also revealed increased E-cadherin expression and decreased vimentin and vascular endothelial growth factor (VEGF) mRNA levels subsequent to the transfection. Bioinformatic analysis further uncovered a specific interaction between MALAT1 and miR-150-3p, indicating elevated MALAT1 expression in breast cancer with a negative correlation to miR-150-3p expression. Furthermore, the overexpression of MALAT1 markedly suppressed miR-150-3p expression, indicating a reciprocal inhibition between the two. Luciferase reporter assays confirmed the specific association between miR-150-3p and MALAT1 at the sequence level. Furthermore, transfection with miR-150-3p mimic inhibited breast cancer cell migration, colony formation, and invasion, along with increased E-cadherin expression and decreased Vimentin and VEGF mRNA levels. Conversely, miR-150-3p inhibitor transfection led to opposing effects, reducing E-cadherin expression, and elevating vimentin and VEGF mRNA levels, while also inhibiting migration, colony formation, and invasion. Functionally, MALAT1 siRNA restrained breast cancer cell proliferation and migration while enhancing cellular apoptosis on administration to breast cancer cells, primarily mediated through miR-150-3p. Our findings delineate MALAT1 as a tumor growth-promoting gene antagonized by miR-150-3p. Building on these insights, this study proposes a potential therapeutic avenue for breast cancer treatment.

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Keywords: lncRNA MALAT1; miR-150-3p; Breast cancer

1. Introduction

Among women, breast cancer is the most common form of cancer. Notably, it boasts a high potential for cure, with curability rates ranging from 70% to 80% for patients in the early stages of the disease and devoid of metastasis.¹ Despite strides made in clinical and epidemiological research, the situation remains challenging for advanced breast cancer patients characterized by distant metastases, which are now considered

untreatable with existing medicines. In 2018 alone, nearly 2.1 million new cases of breast cancer emerged worldwide, resulting in approximately 627,000 deaths attributable to the disease.² The diagnosis of breast cancer largely depends on a combination of clinical evaluation, imaging tools such as mammography or ultrasound, and biopsy needle operations.^{3,4} Although screening techniques such as mammography and serum tumor markers are gaining traction, the insufficient healthcare infrastructure in many areas hinders early breast cancer diagnosis. Therefore, it is essential to delve deeper into molecular pathways and explore novel non-invasive biomarkers to improve our comprehension and detection capacities in the breast cancer context.

The present study examined long non-coding RNAs (lncRNAs), a specific category of non-coding RNA characterized by their length, which surpasses 200 nucleotides. These lncRNAs have emerged as important factors in a wide range of biological processes, including cell death, cell growth, cell migration, resistance to drugs, and cell differentiation.⁵⁻⁷ One notable lncRNA, MALAT1, is encoded by a gene located on chromosome 11q13.1. Initially identified during research on genes linked to hormone therapy resistance in breast cancer cells, MALAT1's expression profile directly correlates with resistance to anti-estrogen treatment.⁸ This phenomenon is notably heightened in several types of cancer, including bladder cancer,⁹ colorectal cancer,¹⁰ and lung cancer,¹¹ wherein MALAT1 enhances tumor cell survival by inhibiting programmed cell death (apoptosis) and promoting cell proliferation and motility. MALAT1 coordinates the initiation of the ErbB2/ErbB3 and Wnt/ β -catenin signaling pathways, fostering resistance against tamoxifen in breast cancer cells,¹² as well as resistance against cisplatin in gastric cancer cells.¹³ Mounting evidence further indicates a connection between heightened MALAT1 levels and unfavorable outcomes in individuals with cancer, highlighting its potential as a prognostic biomarker.¹⁴⁻¹⁶ The complex molecular interactions involving MALAT1, which play a crucial role in carcinogenesis, metastasis, and endocrine resistance in breast cancer, remain incompletely elucidated. This study investigated the expression levels of MALAT1 in breast cancer cell lines and assessed its influence on breast cancer cell survival, migration, invasion, and resistance to treatment. The findings indicate that MALAT1's role in sequestering miR-150-3p leads to an upregulation of CCR7 and ABCB1 expression, thereby promoting the progression of breast cancer. The study emphasizes the carcinogenic properties of MALAT1 and proposes it as a potential therapeutic target to mitigate disease progression and medication resistance in breast cancer.

2. Materials and methods

2.1. Cell culture

The MCF7 and MDA-MB-231 cell lines were generously provided by the Institute of Biochemistry and Cell Biology, Chinese Academy of Sciences in Shanghai, China. These cell lines were cultured in DMEM medium (Gibco BRL, USA) supplemented with 10% fetal bovine serum (FBS, HyClone, USA) to support their growth and viability. Subsequently, the cells were incubated in a controlled environment at 37°C, 5% CO₂, and maintained in a humidified atmosphere to ensure optimal conditions for their growth and survival throughout the experimental procedures.

2.2. RNA isolation and real-time polymerase chain reaction (RT-PCR)

RNA extraction from the cells was performed utilizing the Trizol reagent (Takara, China), following the manufacturer's instructions. Subsequently, first-strand cDNA was synthesized from the extracted RNA samples using the First Strand cDNA Synthesis Kit (Takara, China). The quantitative RT-PCR (qRT-PCR) was conducted on an ABI 7500 system (Applied Biosystems, USA) using the standard SYBR-Green PCR kit. The β -actin gene served as an internal control for each sample. The following primer sequences were utilized:

1. MALAT1¹⁷

Forward primer: 5'-ATCTGCAAAACAAAACCCCT-3'

Reverse primer: 5'-GTCTCCGAAGACACAGAGACCT-3'

2. β -actin

Forward primer: 5'-GGGAAATCGTGCGTGACATTAAG-3'

Reverse primer: 5'-TGTGTTGGCGTACAGGTCTTTG-3'

The qRT-PCR experiments were performed in triplicate to ensure accuracy and reproducibility. RNA expression levels were assessed using the comparative Ct technique ($\Delta\Delta$ Ct method), allowing for the measurement of changes in gene expression relative to the internal reference gene β -actin.

2.3. Small interfering RNA (siRNAs) transfection

A collection of siRNAs targeting MALAT1 (ON-TARGETplus SMARTpool, Dharmacon, USA) was used for transfection, the process of introducing foreign genetic material into cells. Transfection was performed using Lipofectamine 3000 (Invitrogen, USA) following the manufacturer's instructions. Cells were cultured in suitable containers to achieve a confluency of approximately 60–70% at the time of transfection. The specific siRNA designed to target MALAT1 was combined with Lipofectamine

3000. Subsequently, the siRNA-Lipofectamine mixture was added to the cell culture medium, and the cells were placed in a controlled environment set at 37°C, 5% CO₂, and appropriate humidity for the designated transfection duration.

Following transfection, cells were cultured for an additional period to allow for suppression of MALAT1 gene expression by the siRNA. The efficacy of siRNA-induced reduction of MALAT1 expression was evaluated using qRT-PCR or Western blotting to measure mRNA or protein levels, respectively. These measurements were compared to those of control or untreated cells. Functional assays might also be performed to assess the phenotypic consequences of MALAT1 knockdown, such as changes in cell proliferation, migration, invasion, or other relevant cellular processes associated with MALAT1 activity.

2.4. Establishing vectors and stable cell lines

Oligonucleotides targeting MALAT1 were designed and synthesized to create specific short hairpin RNAs (shRNAs), namely, si-MALAT1 (5'-GCAGAGGCATTTTCATCCTT-3') and si-control (5'-TTCTCCGAACGTGTCACGT-3'). An annealing process was employed to generate double-stranded DNA fragments from the designed oligonucleotides. Subsequently, these fragments were ligated into the shRNA expression vector pGPH1/Neo (GenePharma, China), following the manufacturer's instructions, to produce shRNA-expressing constructs targeting MALAT1.

Both MCF7 and MDA-MB-231 cells were seeded in appropriate culture containers and transfected on reaching 60–70% confluency. The MALAT1 shRNA expression vectors or scrambled shRNA (negative control) were transfected into cells using Lipofectamine 3000 (Invitrogen, USA) as per the manufacturer's protocol. Subsequent to transfection, cells were allowed a recovery period before the selection process.

Transfected cells were then subjected to selection pressure using neomycin (G418) at a concentration of 1000 µg/mL. After approximately 4 weeks, cells were continuously monitored for growth and viability during neomycin selection. Neomycin-resistant cells harboring scrambled shRNA or integrated MALAT1 expression vectors were chosen and propagated for further characterization.

The effectiveness of MALAT1 knockdown was assessed using qRT-PCR or Western blotting to confirm reduced mRNA or protein levels, respectively. Functional experiments were conducted to evaluate phenotypic changes associated with MALAT1 knockdown, including alterations in cell proliferation, migration, invasion, and other cellular behaviors linked to MALAT1 activity. The

MCF-10A and SaOS₂ cell lines were used as controls in this study. This approach aimed to establish stable cell lines with diminished MALAT1 expression to investigate its role in breast cancer cellular behavior and its potential as a therapeutic target.

2.5. Cell proliferation assay

A cell suspension was prepared by diluting cells to a concentration of 2.0×10^3 cells/mL in an appropriate culture medium. Subsequently, 100 µL of the cell suspension was dispensed into individual wells of a 96-well plate.

The plate was then incubated at 37°C with 5% CO₂ to support cell adhesion and growth. At 24, 48, and 72 h post-plating, the Cell Counting Kit-8 (CCK-8, Sigma-Aldrich, USA) reagent was added to each well following the manufacturer's instructions. The plate was incubated in the incubator at 37°C for an appropriate duration specified by the manufacturer to allow living cells to metabolically break down the CCK-8 reagent. After the designated incubation period, absorbance at 450 nm was measured using a plate reader. Absorbance readings were taken at each time point (24, 48, and 72 h) for all wells. Mean absorbance values were then calculated from triplicate wells at each time point. Cell proliferation curves were generated by plotting the absorbance at 450 nm against the corresponding time intervals. To ensure reliability and reproducibility, three replicate tests were conducted. Appropriate controls, including blank and negative controls, were incorporated into the experiment. This assay provided crucial insights into long-term cell survival and growth rates based on their metabolic activity, aiding in the evaluation of different experimental conditions or treatments on cell growth kinetics.

2.6. Wound healing assay

Following the scratch induction, the cells were maintained in a culture medium containing 1% FBS to reduce cell proliferation and promote cell motility, thereby facilitating wound healing. Photographs of the wound area were captured at two specific time points: (i) immediately (0 h) and (ii) 48 h of incubation after the scratch, using an IX81 microscope (Olympus, Japan) at a magnification of 100×. The migration rate was quantitatively assessed and analyzed using ImageJ software. The software was employed to measure and quantify the closure or healing of the scratched region over the 48-h incubation period. This analysis facilitated the evaluation of cellular migration and wound closure dynamics in the tested cell lines.

2.7. Transwell assay

The migration and invasion capacities of MCF7 and MDA-MB-231 cells were evaluated using the Transwell

assay. Briefly, approximately 24 h post-transfection, cells were harvested from the culture plates and suspended in 200 μ L of serum-free medium. For the migration assay, 2.0×10^4 cells were seeded into the upper chamber of Transwell inserts (Corning, USA) without Matrigel coating. For the invasion assay, 3.0×10^4 cells were seeded into the upper chamber of Transwell inserts pre-coated with Matrigel (BD Biosciences, USA), which mimics the extracellular matrix. In both the migration and invasion assays, 600 μ L of complete culture medium containing chemoattractants was added to the lower chambers of the Transwell as a stimulus for cell migration.

Following cell seeding, the Transwell chambers were incubated for 48 h under suitable culture conditions. After incubation, non-migrating or non-invading cells on the upper side of the membrane were gently removed. Subsequently, the cells that migrated or invaded through the membrane were fixed, immobilized, and stained with a 0.1% crystal violet solution (Beyotime, China). Images of the migrated or invaded cells were captured using an IX81 microscope (Olympus, Japan) at a magnification of 100 \times . The numbers of migrated or invaded cells were counted from multiple fields of view on each membrane using the microscope. This assay allowed for the assessment and quantification of cell migration and invasion capabilities in response to experimental conditions or treatments.

2.8. Luciferase reporter assay

The GLI luciferase reporter plasmid was introduced into MALAT1-knockdown or control MCF7/MDA-MB-231 cells through Lipofectamine-mediated gene transfer, following the manufacturer's instructions.

The relative luciferase activity was assessed 48 h post-transfection using the Dual-Glo Luciferase Reporter Assay System (Promega, USA), following the provided manufacturer's protocols.

Luciferase activity, indicative of the transcriptional activity of the GLI reporter, was quantified using a luminometer. To account for variations in transfection efficiency and cell count, measurements were standardized by normalizing to the Renilla luciferase activity. This normalization ensured accurate comparative analysis.

The relative luciferase activity derived from the assay was computed and utilized for data analysis. This analysis provided crucial insights into the transcriptional activity of GLI in both MALAT1-knockdown and control MCF7 cells. The experiment facilitated the assessment of the impact of MALAT1 depletion on GLI-mediated transcriptional regulation.

2.9. Statistical analysis

Various statistical tests were applied, including Wilcoxon signed-rank test, Mann-Whitney test, Chi-squared test, log-rank test, *t*-test, and Pearson correlation. The selection of these tests was based on the characteristics of the data, variables involved, and study objectives. Statistical analyses were conducted using SPSS 18.0 software. These tests were performed to assess differences and associations between groups. *P*-values were computed for each analysis to determine significance, with the significance level set at *P* < 0.05 (95% confidence). Selecting appropriate tests and determining the significance level were crucial for meaningful interpretation of the results. The findings were analyzed and interpreted based on the statistical outcomes, ensuring robust conclusions were drawn from the data.

3. Results

3.1. MALAT1 expression patterns in breast cancer cell lines

Examination of MALAT1 expression in MCF7 and MDA-MB-231 breast cancer cell lines exhibited distinct expression patterns compared to normal MCF-10A breast cells (Figure 1). MALAT1 expression was significantly higher in both MCF7 and MDA-MB-231 cell lines compared to MCF-10A cells, indicating its potential relevance in breast cancer, consistent with known oncogenic roles of MALAT1 in various cancers. The conspicuous upregulation of MALAT1 in these cancerous cell lines suggests its potential as a diagnostic biomarker or therapeutic target. This significant elevation justifies further investigation into the specific pathways through which MALAT1 contributes to the aggressive behavior of these breast cancer cells. Understanding the implications

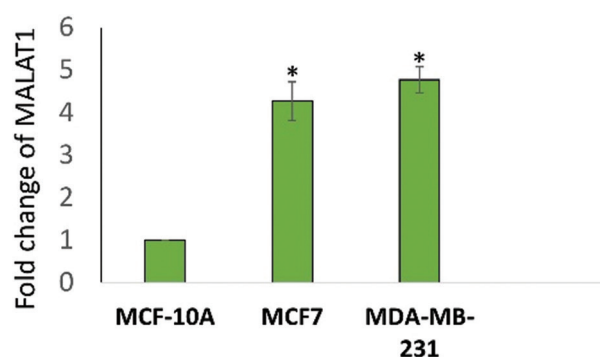


Figure 1. MALAT1 expression in breast cancer cell lines. The qRT-PCR results demonstrate the expression levels of MALAT1 in the MCF-10A, MCF7, and MDA-MB-231 cells.

Notes: The data are expressed as mean \pm standard deviation. Statistical significance is indicated by the notation **P* < 0.05.

Abbreviation: qRT-PCR: Quantitative real-time polymerase chain reaction.

of MALAT1 overexpression might lead to tailored treatments aimed at mitigating its oncogenic effects and improving patient outcomes. Further, exploration of MALAT1's downstream pathways in breast cancer cells could offer crucial insights into its precise function in disease progression.

3.2. Suppression of MALAT1 in breast cancer cells in mitigating malignant attributes and chemoresistance

An investigation was carried out to assess the impact of MALAT1 knockdown on various malignant characteristics of breast cancer cells. Using MALAT1 siRNAs (si-1, si-2, and si-3), a notable reduction in MALAT1 expression was achieved, with si-1 displaying the most prominent suppression (Figure 2A and E). This decrease in MALAT1 expression significantly affected the migratory and invasive abilities of both MCF7 and MDA-MB-231 cells, as demonstrated in the Transwell assays (Figure 2B-D and F-H). Moreover, the wound healing assay revealed a considerable impediment to the migration of breast cancer cells on diminishing MALAT1 expression (Figure 2D and E). Notably, treatment with si-1 notably reduced the number of colonies formed by breast cancer cells (Figure 2F). Furthermore, MALAT1

suppression notably reduced the 50% inhibitory concentration (IC_{50}) of doxorubicin in both MCF7 and MDA-MB-231 cells (Figure 2H). In addition, the levels of ABCB1 and MALAT1 expression were considerably elevated in doxorubicin-resistant MCF7/doxorubicin and MDA-MB-231/doxorubicin cells compared to their original counterparts. Overall, these findings highlight that MALAT1 suppression significantly impedes cell migration, invasion, and chemotherapy resistance, as well as potentially inhibits the transition from epithelial to mesenchymal cells in breast cancer. These results underscore the potential therapeutic relevance of targeting MALAT1 to combat the malignant progression and drug resistance mechanisms in breast cancer.

3.3. Reduction of the expression of miR-150-3p by MALAT1

In our study investigating MALAT1's impact on breast cancer progression, we explored its potential interaction with specific microRNAs (miRNAs). Our analysis using StarBase V3.0 revealed a potential binding relationship between MALAT1 and miR-150-3p (Figure 3A). Subsequent luciferase reporter assays performed in MCF7 and MDA-MB-231 cells indicated that increasing miR-150-3p levels notably reduced luciferase activity in cells carrying the

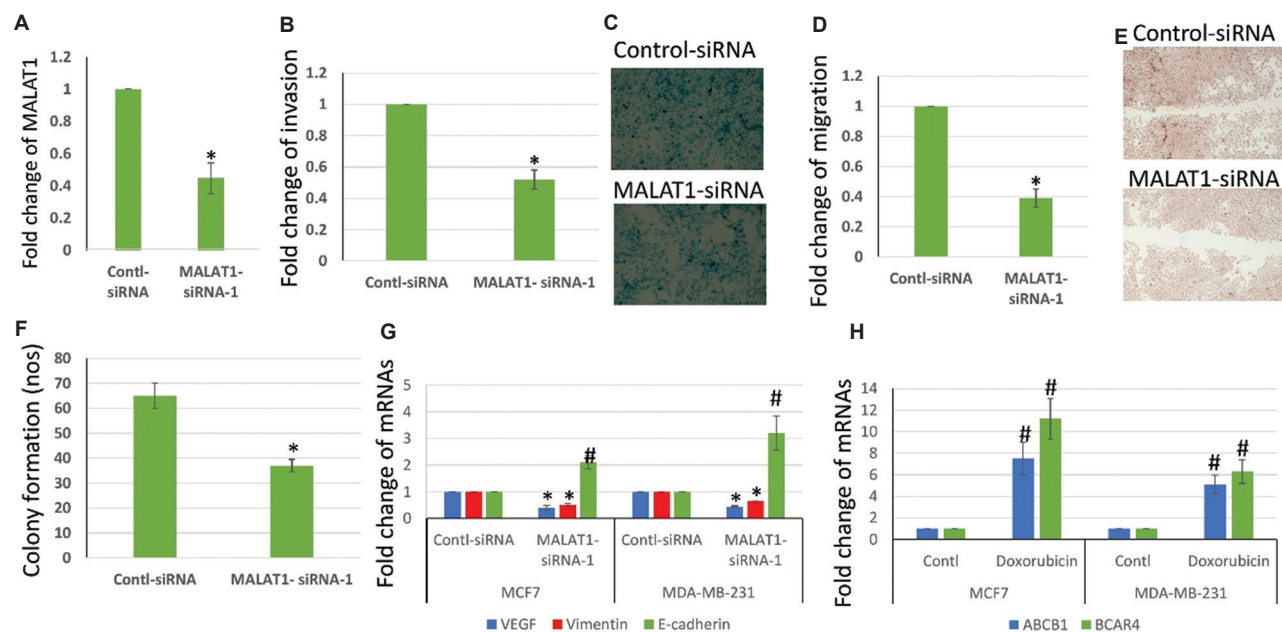


Figure 2. MALAT1 inhibition suppresses cell migration, invasion, and doxorubicin resistance in both MCF7 and MDA-MB-231 cells. MALAT1 siRNA was used to transfect MCF7 cells, and the relative expression of MALAT1 was analyzed using qRT-PCR (A). The expression levels were compared to the control group. Transwell tests were conducted to demonstrate the effect of MALAT1 suppression on cell migration and invasion in MCF7 cells (B-H). The expression of ABCB1 and metastasis-related genes (E-cadherin, Vimentin, and VEGF) in MCF7 and MDA-MB-231 cells transfected with MALAT1 siRNA was analyzed using the qRT-PCR technique and compared to the control group.

Notes: The data are expressed as mean \pm standard deviation of three replicates. *Indicates statistically decreased, and # indicates statistically increased compared to control. Abbreviations: qRT-PCR: Quantitative real-time polymerase chain reaction; VEGF: Vascular endothelial growth factor.

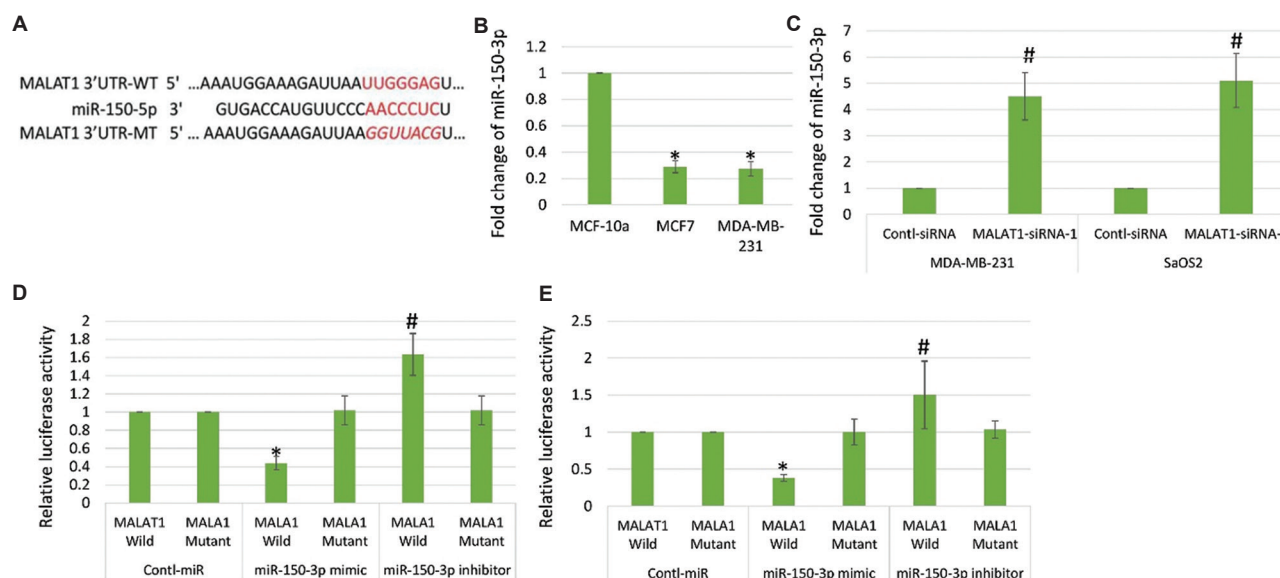


Figure 3. The interplay between MALAT1 and miR-150-3p, as well as the expression of MALAT1 in breast cancer cells. (A) The binding sites of MALAT1 and miR-150-3p, as predicted by StarBase V3.0, are presented. Highlighted are the binding locations that have undergone mutation, provided for reference. The luciferase reporter test was conducted in MCF7 and MDA-MB-231 cells (B-E). Cells were simultaneously transfected with pRL-TK containing either the wildtype or mutant binding site, in addition to the miR-150-3p mimic or the miR-150-3p inhibitor. Luciferase activity was measured 48 h after transfection. The expression levels of miR-150-3p in MCF7 and MDA-MB-231 cells were measured using a qRT-PCR assay after transfection with MALAT1 siRNA and a control.

Notes: The results are expressed as mean \pm standard deviation. *Indicates statistically decreased, and # Indicates statistically increased compared to control. Abbreviation: qRT-PCR: Quantitative real-time polymerase chain reaction.

normal MALAT1 construct but had no effect on those with a mutated MALAT1 construct (Figure 3A-E). Conversely, reducing miR-150-3p expression significantly increased luciferase activity in cells with the wildtype MALAT1 construct but had no impact on cells with the mutated MALAT1 construct. Further, investigation demonstrated that suppressing MALAT1 led to a considerable elevation in miR-150-3p expression in both MCF7 and MDA-MB-231 cells (Figure 3A). In addition, compared to the hFOB 1.19 normal osteoblast cell line (SaOS2), the levels of miR-150-3p were notably diminished in various MCF7 and MDA-MB-231 cells (Figure 3A-E). These findings collectively indicate a direct interaction between MALAT1 and miR-150-3p, suggesting MALAT1's role as a suppressor of miR-150-3p expression in breast cancer.

This newly identified regulatory link between MALAT1 and miR-150-3p sheds light on a potentially crucial pathway involved in breast cancer development. Not only does this discovery enhance our understanding of the underlying molecular mechanisms but it also presents a promising avenue for therapeutic intervention by targeting the MALAT1-miR-150-3p interaction. Further, elucidation of this interaction may offer innovative strategies for therapeutic interventions in breast cancer aimed at disrupting this specific regulatory mechanism.

3.4. Enhanced migration, invasion, and chemotherapy resistance in breast cancer cells by MALAT1-mediated suppression of miR-150-3p expression

On transfecting MALAT1 siRNA along with a miR-150-3p inhibitor into MCF7 and MDA-MB-231 cells, we observed a reversal of the diminished colony formation triggered by MALAT1 silencing in breast cancer cells (Figure 4A and B). Cotransfection with the miR-150-3p inhibitor counteracted the reduced migration and invasion capabilities induced by MALAT1 siRNA in MCF7 and MDA-MB-231 cells, as evidenced by wound healing and Transwell assays (Figure 4C). Furthermore, inhibition of MALAT1 decreased cell survival and reduced doxorubicin concentration needed to impede cell growth, which were successfully restored by the miR-150-3p inhibitor in both MCF7 and MDA-MB-231 cells (Figure 4D). Conversely, overexpression of MALAT1 led to increased cell survival and reduced doxorubicin IC₅₀, effects mitigated by the miR-150-3p mimic in both cell lines.

Remarkably, the downregulation of ABCB1, Vimentin, and vascular endothelial growth factor (VEGF), coupled with the upregulation of E-cadherin expression due to MALAT1 suppression, was reversed in MCF7 and MDA-MB-231 cells by the miR-150-3p inhibitor (Figure

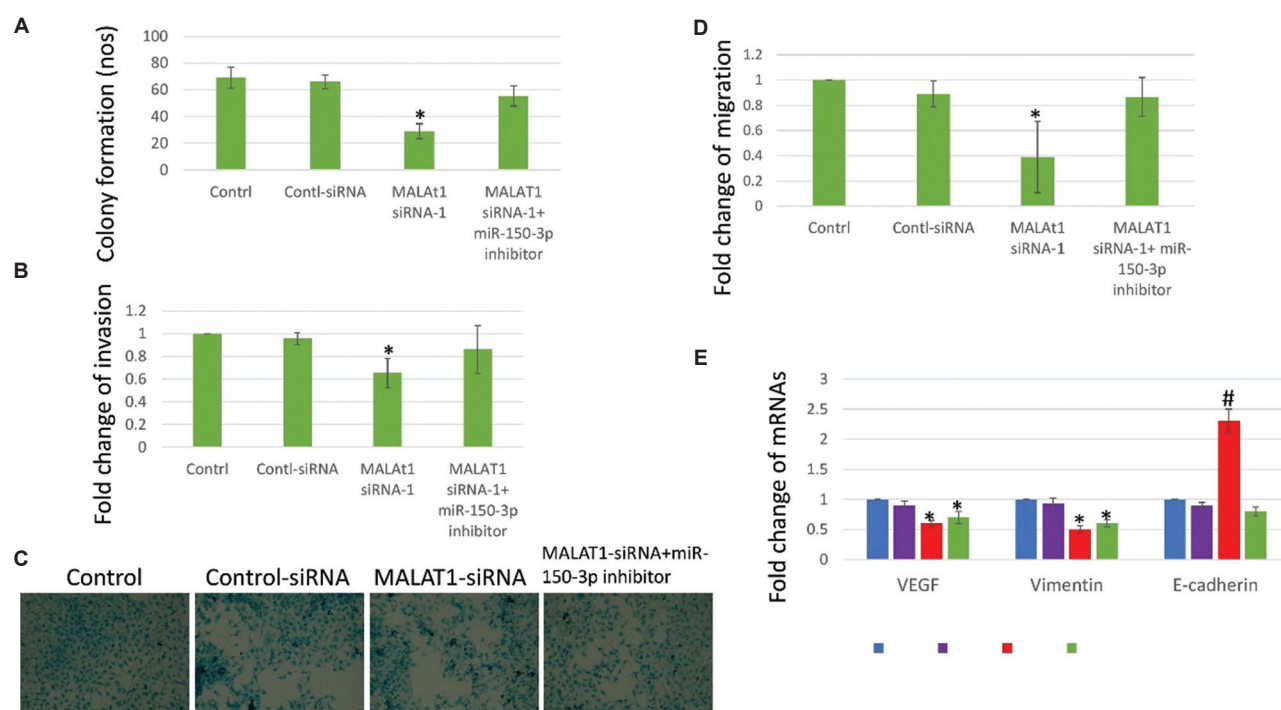


Figure 4. MALAT1 enhances the migratory, invasive, and chemoresistant abilities of MCF7 and MDA-MB-231 cells by reducing the expression of miR-150-3p. The impact of si-MALAT1 and/or miR-150-3p inhibitor transfection on cell colonies (A) in MCF7 cells was assessed using a colony formation assay. (B and C) The wound healing experiment demonstrates cell migration after transfection with either the control, si-MALAT1, or the combination of si-MALAT1 and miR-150-3p inhibitor. (D) Transwell assay was conducted to demonstrate the effects of control, si-MALAT1, or si-MALAT1 combined with miR-150-3p inhibitor transfection on the migratory and invasive abilities of MCF7 cells. (E) The expression levels of E-cadherin, Vimentin, and VEGF in MDA-MB-231 cells were analyzed using qRT-PCR after transfection with si-MALAT1 and/or miR-150-3p inhibitor.

Notes: The results are expressed as mean \pm standard deviation. The notation * indicates statistically decreased, and # indicates statistically increased compared to control.

Abbreviations: qRT-PCR: Quantitative real-time polymerase chain reaction; VEGF: Vascular endothelial growth factor.

4E). These findings underscore the substantial role of MALAT1 in promoting the migration, invasion, and chemotherapy resistance of breast cancer cells, partly mediated through miR-150-3p regulation. The observed reversal of MALAT1 siRNA-induced effects following cotransfection with the miR-150-3p inhibitor underscores the pivotal involvement of miR-150-3p in driving MALAT1-induced malignancy traits. The intricate interplay between MALAT1 and miR-150-3p suggests the potential utility of targeting this axis therapeutically to impede the aggressive behavior of breast cancer cells. A deeper understanding of this regulatory mechanism could open new avenues for developing targeted therapies aimed at disrupting this specific molecular pathway in breast cancer.

3.5. Regulation of breast cancer cell migration and invasion by MiR-150-3p through vimentin and VEGF signaling

The primary objective of this study was to assess the influence of miR-150-3p, a crucial regulatory

molecule, on breast cancer cell migration, invasion, and chemotherapy resistance. We introduced miR-150-3p mimic and inhibitor into MDA-MB-231 cells to investigate the impact of altered miRNA activity. Colony formation assays demonstrated reduced colony numbers in MDA-MB-231 cells transfected with the miR-150-3p mimic, while those transfected with the miR-150-3p inhibitor exhibited notably higher colony numbers compared to control cells (Figure 5A). Moreover, heightened miR-150-3p expression significantly inhibited cell migration and invasion, whereas miR-150-3p suppression promoted these activities (Figure 5B and C). CCK8 tests revealed decreased cell viability and reduced doxorubicin IC₅₀ in MDA-MB-231 cells transfected with the miR-150-3p mimic, whereas the miR-150-3p inhibitor elicited the opposite effect (Figure 5D). These results emphasize the suppressive role of miR-150-3p in breast cancer cell migration, invasion, and chemotherapy resistance. Additional investigation in MDA-MB-231 cells indicated that heightened miR-150-3p levels led to decreased expression of ABCB1, Vimentin, and VEGF,

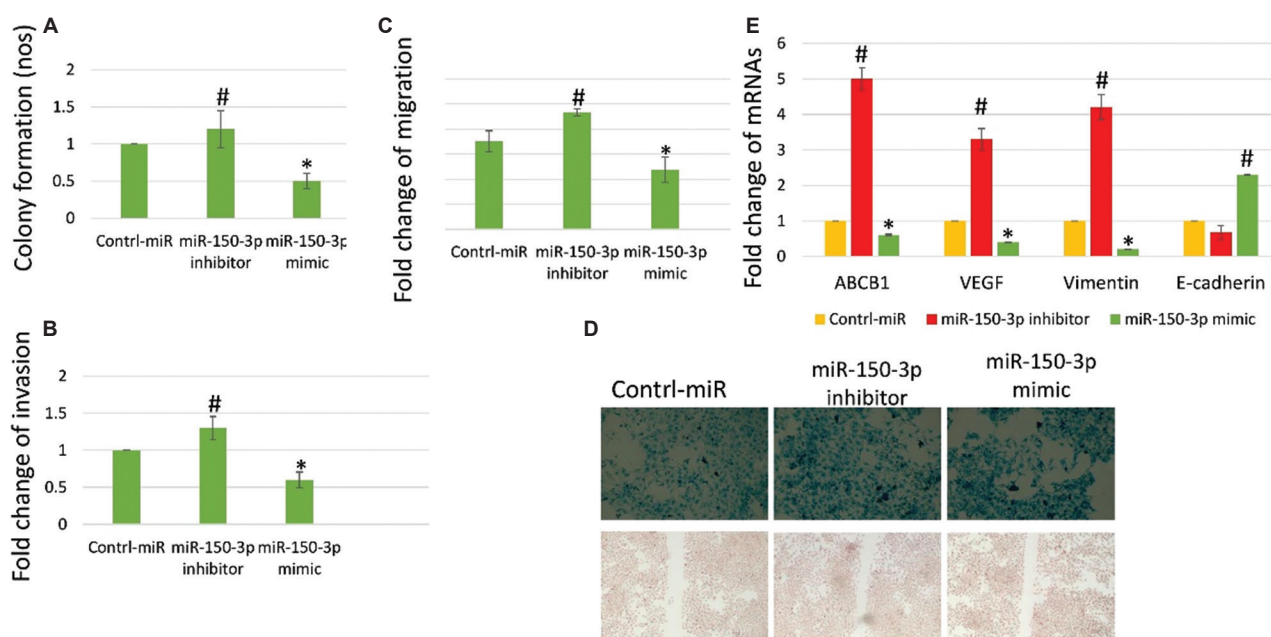


Figure 5. The effects of overexpressing or inhibiting miR-150-3p on cell migration, invasion, and resistance to doxorubicin in MDA-MB-231 were investigated. (A) The colony formation experiment demonstrates the effect of modified miR-150-3p expression on MDA-MB-231 cells. (B and C) The wound healing experiment demonstrates cell migration using a miR-150-3p mimic, a miR-150-3p inhibitor, and their corresponding control samples. (C and D) The modified miR-150-3p expression was assessed using a Transwell test to measure cell invasion. (E) The qRT-PCR results demonstrate the expression levels of E-cadherin, Vimentin, and VEGF after alterations in miR-150-3p expression. The expression levels of E-cadherin, Vimentin, and VEGF were measured using qRT-PCR with manipulated miR-150-3p expression.

Notes: The results were expressed as mean \pm standard deviation from three replicates. *Indicates statistically decreased, and # indicates statistically increased compared to control.

Abbreviation: qRT-PCR: Quantitative real-time polymerase chain reaction; VEGF: Vascular endothelial growth factor.

while increasing E-cadherin expression (Figure 5E). Conversely, miR-150-3p suppression yielded contrasting effects on these proteins, underscoring its significant influence on proteins associated with cellular behavior. MALAT1 suppression in MDA-MB-231 cells effectively restored Vimentin and VEGF downregulation, a reversal observed when cotransfecting with the miR-150-3p inhibitor (Figure 5E). These findings indicate miR-150-3p's pivotal role in regulating Vimentin and VEGF production post-MALAT1 suppression, suggesting its involvement in governing breast cancer cell migration and invasiveness.

4. Discussion

Breast cancer primarily affects individuals in older age groups, particularly postmenopausal women.¹ lncRNAs play a crucial role in regulating gene expression, thereby modulating various cellular functions. Their interactions with miRNAs contribute to the complexity of cellular regulatory networks.^{14,16-22} Several studies have identified altered expressions of several lncRNAs in breast cancer, such as MALAT1, MEG3, HUMT, SNHG25, STK4, and KIAA0087,

which correlate with diverse cellular activities.^{4,23-28} Our investigation observed increased MALAT1 levels in breast cancer cells, particularly associated with advanced disease stages. Blocking MALAT1 significantly reduced cell viability, migration, and invasion in breast cancer cells, indicating its crucial role in fostering malignancy. Moreover, elevated MALAT1 expression in doxorubicin-resistant cells, along with heightened ABCB1 levels, underscores its association with drug resistance. Inhibition of MALAT1 increased breast cancer cell sensitivity to doxorubicin by reducing ABCB1 expression, suggesting MALAT1 as a potential therapeutic target.

Moreover, miR-150-3p, identified as a MALAT1-interacting miRNA, exhibits tumor-suppressive functions and may play a regulatory role in breast cancer. Our findings demonstrate a reciprocal relationship between MALAT1 and miR-150-3p expression levels. Knockdown of MALAT1 upregulated miR-150-3p, suggesting a regulatory link in breast cancer. Suppression of miR-150-3p reversed the impact of MALAT1 inhibition, while heightened MALAT1 levels contributed to doxorubicin resistance, both effects being counteracted by altering miR-150-3p expression.

Our investigation into miR-150-3p revealed its pivotal role in breast cancer progression, influencing cell survival, migration, invasion, and drug resistance by regulating key proteins such as ABCB1, Vimentin, VEGF, and E-cadherin. In addition, a systematic review of the therapeutic potential of PD-L1-inhibiting miRNAs for triple-negative breast cancer (TNBC) has been conducted. The review explores the role of single-cell sequencing-guided biomimetic delivery strategies in enhancing treatment efficacy, offering valuable insights for precision medicine approaches in TNBC therapy.²⁹ Our results highlight MALAT1's role in promoting breast cancer metastasis and chemotherapy resistance through interactions with miR-150-3p. These findings complement previous research that has demonstrated MALAT1's contribution to breast cancer progression through epigenetic regulation and chemokine signals.¹⁸ The intricate interplay between MALAT1, miR-150-3p, and crucial biological pathways underscores their collective impact on aggressive behavior and resistance mechanisms in breast cancer cells. Understanding these interactions may offer therapeutic prospects to mitigate breast cancer progression and drug resistance.

5. Conclusion

Our investigation elucidates the considerable oncogenic impact of MALAT1 in breast cancer. A thorough analysis unveiled its multifaceted roles, significantly influencing the aggressive traits of this malignancy. Our findings underscore MALAT1's pivotal role in driving breast cancer cell migration and invasion, particularly through its interaction with miR-150-3p. This interaction remarkably enhances the metastatic potential of breast cancer cells, shedding light on a newly discovered mechanism underlying their aggressive behavior.

Moreover, our study highlights MALAT1's regulatory influence on ABCB1, a key contributor to chemotherapy resistance in breast cancer. MALAT1 notably amplifies chemotherapy resistance by upregulating ABCB1 expression, posing a significant challenge in effectively treating this cancer type. These results bear substantial implications for developing therapies aimed at curbing cancer spread and drug resistance in breast cancer. Identifying MALAT1 as a crucial player in these processes positions it as an appealing therapeutic target. Strategies aimed at modulating MALAT1 expression or its downstream effects could offer innovative avenues for therapeutic intervention, offering promising approaches to combat breast cancer metastasis and chemotherapy resistance. Essentially, our data underscore MALAT1's vital role in orchestrating the aggressive behavior of breast cancer cells. Its multifaceted involvement in promoting metastasis and inducing chemotherapy resistance renders it

an encouraging and feasible therapeutic target to confront the challenges posed by breast cancer progression, offering a potential avenue to enhance treatment strategies and patient outcomes against this formidable disease.

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Conflict of interest

The authors declare that they have no competing interests.

Author contributions

Conceptualization: All authors

Formal analysis: All authors

Investigation: Krishnamoorthy Vignesh

Methodology: All authors

Writing – original draft: Krishnamoorthy Vignesh

Writing – review & editing: Krishnamoorthy Vignesh

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data

Data will be made available with a reasonable request.

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